EGFR Gene Mutation Analysis in Non-Small Cell Lung Cancer Using cobas® Assay in FFPE and Plasma Specimen Types

:: Background

The FDA approved cobas® EGFR Mutation Test v2 is a real-time PCR test for the qualitative detection of defined mutations of the EGFR gene in DNA derived from formalin-fixed paraffin-embedded (FFPE) or plasma human non-small cell lung cancer (NSCLC) patients. The test is intended to aid in identifying patients with NSCLC whose tumors have defined EGFR mutations and for whom safety and efficacy of a drug have been established. In this study, we evaluated the clinical and analytical performance features of the assay.

:: Methods

Genomic DNA was isolated from the tumor specimens using the cobas® DNA Sample Preparation Kit. Mutation detection is achieved through real-time PCR analysis on the cobas® 480 analyzer. DNA from NSCLC specimens were used to evaluate accuracy, repeatability, reproducibility and analytical sensitivity of the assay.

The cobas® EGFR Mutation Test v2 has been offered as a clinical test in LabCorp. Of the 389 FFPE specimens tested, 53.47% were negative, 42.67% were positive with 1-3 EGFR mutations. 27.50% specimens had one variant, 11.31% contained exon 19 deletion/790M variants, 2.83% had T790M/L858R variants, and 1.03% had 3 variants or 2 other variants. Results could not be obtained in 3.85% specimens due to specimen degradation or low DNA yield. Of the 513 plasma specimens tested, 59.84% were negative, 39.96% were positive with 1-3 EGFR mutations. 26.12% specimens had one variant, 7.4% contained exon 19 deletion/790M variants, 5.46% had T790M/L858R variants, and 0.98% had 3 variants or 2 other variants. Results could not be obtained in 1 (0.19%) specimen. There was no difference in the mutation rate and distribution between males and females.

The higher detection sensitivity of the cobas® platform has resulted in the higher mutation detection rate than other platforms.

:: Results

Of the specimens tested during validation, 20 FFPE specimens with known mutations in EGFR had cobas® results that were 90% concordant and 20 plasma specimens were 100% concordant. The lower concordance in FFPE specimen type was due to borderline positive specimens, specimen degradation and detection sensitivity difference between the two platforms. Repeatability was 90% concordant for FFPE specimens and 100% for plasma specimens; reproducibility was 100% concordant for both FFPE and plasma specimens. The lower concordance for FFPE repeatability was due to borderline positive specimens. This assay can detect 5% of mutant DNA in a background of wild type genomic DNA when the input DNA is 50ng for FFPE specimens and 100 copies for plasma specimens.

:: Conclusions

The cobas® EGFR Mutation Test v2 is a robust, reproducible, sensitive, and fast assay for molecular diagnostic utilization in NSCLC using FFPE or plasma specimen types.

:: References

1. cobas® EGFR Mutation Test v2 Package Insert. Rev. 3.0 2016.